VALIDITY OF CARBON TETRACHLORIDE-INDUCED LIVER DAMAGE AND NEPHRECTOMY AS BIOLOGICAL PROCEDURES FOR DETERMINING FATE AND EXCRETION OF ANTICONVULSANT DRUGS

by

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I. GENERAL INTRODUCTION

For many years pharmacologists have been interested in the role of the liver and the kidney in the fate and excretion of anticonvulsant drugs. Much information on this subject has been obtained by the application of chemical and biological methods to the study of the detoxication of anticonvulsant drugs in liver-damaged or bilaterally nephrectomized animals. Most of the early studies were of a chemical type and employed either gravimetric or colorimetric procedures (Fisher and von Mering, 1904; Herwick, 1933; Koppanyi et al., 1933; Kozelka and Tatum, 1937; Kozelka et al., 1939; Raventós, 1946; and others). Because of the many manipulations required by both methods, much of the drug was generally lost and, consequently, the results obtained with these technics tended to be more qualitative than quantitative. However, new and better chemical procedures have now been devised. As a result, the majority of more recent experiments employ highly specific spectrophotometric, radioactive isotopic, and stable isotopic methods (Hellman et al., 1943; van Dyke et al., 1947; Gould et al., 1949; Roth et al., 1949; Kahn, 1950; Goldbaum, 1952; Broughton, 1956; Noach et al., 1957; and others). Nevertheless, there are clinically useful and candidate anticonvulsant drugs for which good quantitative chemical procedures have not as yet been perfected.

The lack of precise chemical methods for the detection of certain agents in various body fluids stimulated the search for biological procedures useful in the study of the fate and excretion of anticonvulsant drugs. Most of these biological procedures are based on the effect of liver damage or nephrectomy on the sleep time of drug-treated animals

(for those anticonvulsants which are sedative, e.g., phenobarbital) or on anticonvulsant activity when assayed versus electrically or chemically induced seizures (Pratt, 1933; Koppanyi et al., 1935, 1936; Cameron and de Sarm, 1939; Dille and Seeburg, 1940; Tatum and Kozelka, 1941; Richards et al., 1946; Moore et al., 1951; Swinyard et al., 1952a,b; Weaver et al., 1952; Swinyard et al., 1954; and Schiffman et al., 1955a). Because of convenience, chemically induced liver damage has been frequently used in the studies cited above.

Many chemical substances are known to be toxic to the liver. For example, arsenic, phosphorus, tannic acid, chloroform, carbon tetrachloride, ethylene dichloride and cinchophen are all known to cause hepatic lesions. Of these several agents, phosphorus and carbon tetrachloride are employed most frequently to induce liver damage in laboratory animals for the study of the fate and excretion of anticonvulsant drugs. Since carbon tetrachloride was the chemical agent employed in the studies conducted at the University of Utah on the fate and excretion of anticonvulsant drugs, a brief consideration of its history and toxic effects is in order.

First prepared in 1849, carbon tetrachloride was used as an anesthetic agent by Simpson, but was soon discarded because of its toxicity (Simpson, 1856; Nunneley, 1867). It then disappeared from therapeutics until Hall (1921) demonstrated that it was effective against hookworm in dogs and recommended its use in man. Following this discovery, there was a rapid increase in the use of carbon tetrachloride in the treatment of hookworm infestations throughout the world. Because of the high toxicity of carbon tetrachloride there soon appeared reports of poisoning and death after its

use. This stimulated the search for pathological and functional abnormalities induced in various organs by carbon tetrachloride. The liver and the kidney have been most extensively investigated and, in this regard, considerable more attention has been directed to the pathological effects induced by this agent than to the functional effects.

With regard to the pathological effects induced by carbon tetrachloride, various workers have observed in both animals and man a marked hepatic centrolobular zonal necrosis (Hall, 1921, 1922; Docherty and Burgess, 1922; Hall and Shillinger, 1923; Lamson et al., 1923; Phelps and Hu, 1924; Lacquet, 1932; Cameron and Karunaratne, 1936; Eschenboenner and Miller, 1946; Moon, 1949; Stowell and Lee, 1950; Drill, 1952; Swinyard et al., 1952a; and others), and a degeneration of renal proximal convoluted tubules, distal convoluted tubules, loops of Henle, and collecting tubules (Meyer and Pessoa, 1923; Lamson et al., 1923; Phelps and Hu, 1924; Smyth et al., 1936; Smetana, 1939; Moon, 1949; Opie, 1950; Ungar, 1951; Jennings and Kearns, 1953; and others). The severity of the hepatotoxic and nephrotoxic action varies with the dose of carbon tetrachloride given, the route of administration, age of the animal, and species employed (Lake, 1922; Meyer and Pessoa, 1923; Lamson et al., 1923; Gardner et al., 1925; Cameron and Karunaratne, 1936; and others).

With regard to the functional abnormalities induced by carbon tetrachloride, several workers have shown that this agent alters the normal function of both the liver and the kidney of various laboratory animals and man. Tests based on the measurement of serum phosphatase levels, prothrombin time, galactose and glucose tolerances, bromsulphalein and

other dye clearances, and the ability of the isolated rat liver to synthesize p-aminohippuric acid from glycine and p-aminobenzoic acid have all indicated a decreased liver function in carbon tetrachloride-treated animals (Okano, 1930; Aubertin et al., 1938; Nagazumi, 1938; Drill and Ivy, 1944; Drill, 1952; and Plaa et al., 1958). Carbon tetrachloride has also been shown to decrease kidney function in humans as measured by inulin, diodrast, and p-aminohippurate clearance tests, and by a decreased ability of the kidney to reabsorb sodium (Corcoran et al., 1943; Sirota, 1949).

Bilateral nephrectomy represents the biological counterpart of liver injury and has been extensively used to study the role of the kidney in the fate and excretion of anticonvulsant drugs (Voss, 1926; Hirschfelder and Haury, 1933; Richards and Everett, 1946; Swinyard et al., 1952a,b; Weaver et al., 1952; Swinyard et al., 1954; Schiffman et al., 1955a,b; Schiffman, 1956; and others).

Since carbon tetrachloride-induced liver damage increased the anticonvulsant activity of the several drugs which they tested, Swinyard and co-workers questioned the validity of carbon tetrachloride-induced liver damage as a useful biological procedure. These workers suggested that lesions produced in organs other than the liver might introduce error. Also, since carbon tetrachloride has a central depressant action similar to chloroform, it was thought that this agent might depress the central nervous system and alter the basal state of the experimental animal.

Bilateral nephrectomy is also open to criticism based largely on the argument that little attention has been paid to the physiological

alterations induced by this procedure. In addition, the accumulation of metabolic products in nephrectomized animals and/or the trauma of the operation might alter the basal state of the animal.

The present investigation represents a systematic laboratory study designed to evaluate the validity of carbon tetrachloride-induced liver damage and bilateral nephrectomy as biological procedures for the determination of the role of the liver and the kidney in the fate and excretion of anticonvulsant drugs. The results obtained provide the basis of this dissertation.

II. STATEMENT OF THE PROBLEM AND WORKING HYPOTHESIS

Carbon tetrachloride-induced liver damage, partial hepatectomy, and bilateral nephrectomy are commonly coupled with bioassay or chemical methods to determine the role of the liver and the kidney in the fate and excretion of drugs. These biological procedures have been used with bioassay methods in our laboratories to determine the role of these organs in the fate and excretion of anticonvulsant drugs (Swinyard et al., 1952a, b; Weaver et al., 1952; Swinyard et al., 1954). It is generally assumed that carbon tetrachloride administration, partial hepatectomy, or nephrectomy does not affect the response of the central nervous system to drugs or to the tests employed to measure drug potency. However, the influence of such biological alterations on the tests employed to measure anticonvulsant activity has been questioned for several reasons. Comparatively little attention has been given to the effect of liver damage or nephrectomy on electrically or chemically induced convulsions. Carbon tetrachloride has a central depressant action and, thus, may decrease susceptibility to experimentally induced seizures. Further, carbon tetrachloride is also known to be nephrotoxic, and its use to produce liver damage might thereby alter the rate at which certain drugs are excreted by the kidney. In view of the above uncertainties it seemed important to test the validity of carbon tetrachloride-induced liver damage, partial hepatectomy, and bilateral nephrectomy as laboratory methods for the determination of the role of the liver and the kidney in the fate and excretion of anticonvulsant drugs.

In order to test the validity of the above-mentioned biological procedures, the effect of carbon tetrachloride-induced liver damage, partial hepatectomy, and nephrectomy on maximal electroshock seizure (MES) pattern, on the ED50s for barbital sodium and pentobarbital sodium as measured by the MES test, and on renal inulin clearance and barbital sodium excretion was determined. Maximal electroshock seizure pattern was selected because the MES test has been used routinely in our laboratory for the study of the role of the liver and the kidney in the fate and excretion of anticonvulsant drugs (Swinyard et al., 1952a,b; Weaver et al., 1952; Swinyard et al., 1954), and because alterations in seizure severity are reflected in the seizure pattern. For example, a decrease in the severity of a maximal seizure is reflected by an increase in the duration of the hindleg tonic-flexor component and a decrease in the hindleg tonic-extensor component (Toman et al., 1946; Swinyard, 1949; Tedeschi, 1955; Laffan et al., 1957). Barbital sodium was selected because more than 90 per cent of a given dose is excreted unchanged by the kidney and it is unaffected by the liver (Maynert and van Dyke, 1949). Pentobarbital sodium was selected because less than 3 per cent of a given dose is excreted by the kidney and more than 97 per cent is detoxified by the liver (Maynert and van Dyke, 1949; Raventós, 1954). Since carbon tetrachloride is known to be nephrotoxic, renal inulin clearance and barbital sodium excretion were used to measure the effect of this agent on renal function.

To facilitate the interpretation of the results a working hypothesis was formulated based on the anticipated effect of carbon tetrachloride-induced liver damage, partial hepatectomy, and nephrectomy on the pattern

of maximal electroshock seizures and on the dose of barbital sodium and pentobarbital sodium which prevented the hindleg tonic-extensor component of maximal seizures in 50 per cent of animals (ED50). In addition, the anticipated effect of liver damage on renal inulin clearance and on the rate of excretion of barbital sodium was also included. If the above-mentioned biological procedures are valid for the determination of the role of the liver and the kidney in the fate and excretion of anticonvulsant drugs, the MES pattern measured in carbon tetrachloride-treated, hepatectomized, or nephrectomized rats should not differ significantly from that measured in control animals. The expected effects of these biological procedures on the ED50s for barbital sodium and pentobarbital sodium are graphically shown in figure 1. In this figure, the ED50s are shown on the ordinate, and the times at which the ED50s were determined are plotted on the abscissa of each small graph. The theoretical ED50s for barbital sodium and pentobarbital sodium, determined at various times after drug administration in normal and experimental rats, are shown by the two solid lines in each of the four small diagrams. As shown by the normal lines, the ED50s are initially high and decline to a minimum as absorption of the drug is completed and the maximum concentration of drug reaches the brain. The ED50s then progressively increase as the drug is inactivated or excreted by the animal. Hence, the initial decrease in the normal curve reflects the mirror image of drug absorption, whereas the subsequent increase in the curve reflects drug inactivation or excretion. The experimental treatment employed (carbon tetrachloride-induced liver damage, hepatectomy, or nephrectomy) can modify this

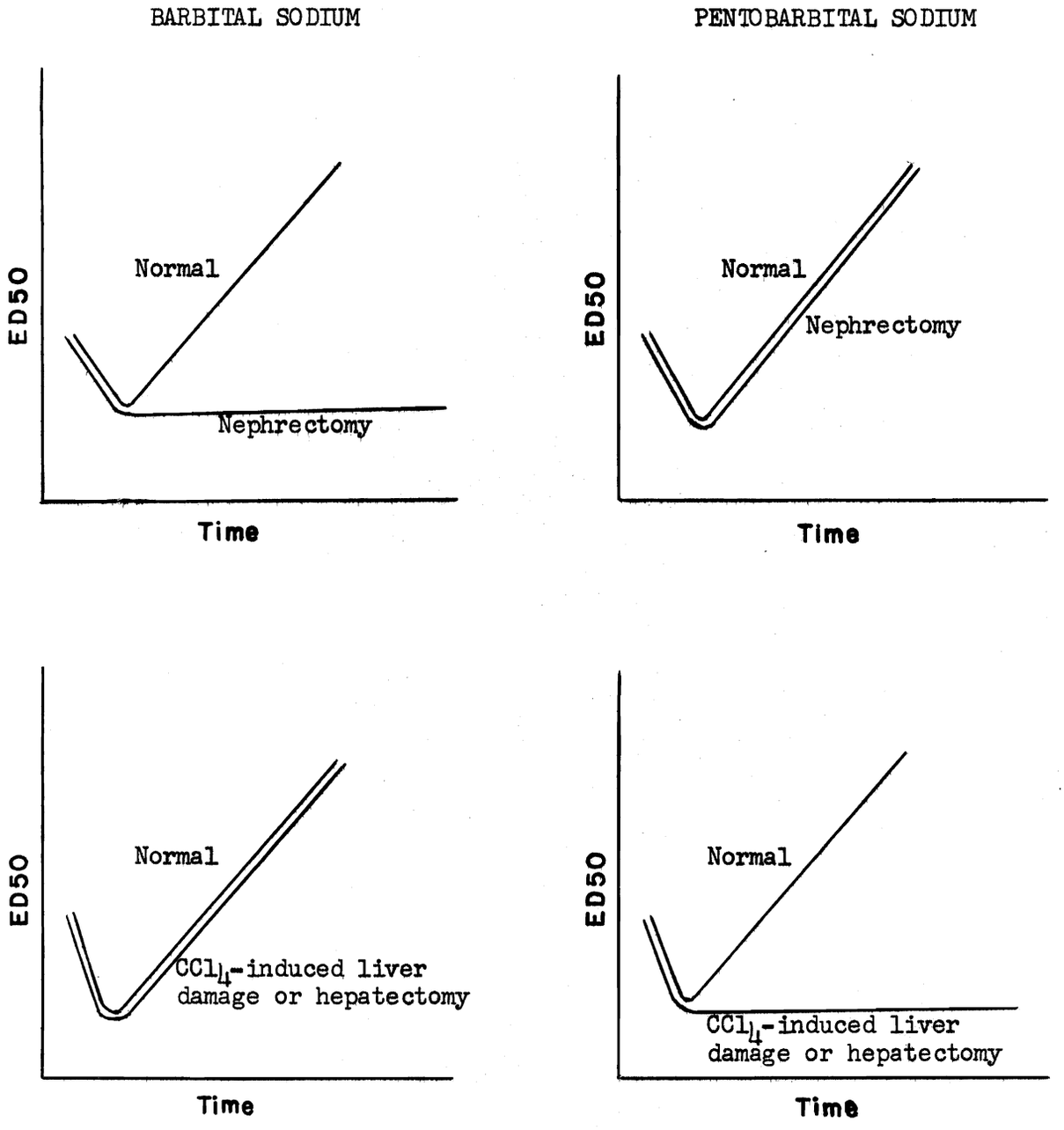

Figure 1. Hypothetical Dose-Time Curves of Barbital Sodium and Pentobarbital Sodium in Carbon Tetrachloride-treated, Hepatectomized, or Nephrectomized Rats.

theoretical normal curve in several ways: It can decrease or increase the slope of the line obtained after drug absorption in experimental rats with respect to that obtained in normal animals, or it can decrease or increase the minimum ED50 with respect to that determined in normal rats. In addition, any combination of the above possibilities may occur. A decrease in the slope of the line for experimental animals with respect to the slope of the line obtained in normal rats may indicate the biological procedure has decreased the rate at which the drug is inactivated or excreted and/or that it has induced a progressive accumulation of metabolic products which act additively with the drug. On the other hand, an increase in the slope of the line obtained in experimental animals with respect to the slope of the line obtained in normal rats may indicate that the experimental procedure has induced a progressive accumulation of metabolic products which act antagonistically with the drug. A decrease in the minimum experimental ED50 indicates an increase in the reactivity of the experimental animal and/or an increase in the rate of absorption relative to inactivation of the drug. Conversely, an increase in the minimum experimental ED50 indicates a decrease in the reactivity of the experimental animal and/or a decrease in the rate of absorption of the drug. Thus, if carbon tetrachloride-induced liver damage and partial hepatectomy are valid procedures for the determination of the role of the liver in the inactivation of anticonvulsant drugs, liver damage should result in almost complete inability of the animal to inactivate or excrete pentobarbital sodium. Hence, the ED50s for this drug after complete absorption should be practically constant with time. Conversely, carbon tetrachloride-induced liver

damage or partial hepatectomy should have no significant effect on the ED50s for barbital sodium. If nephrectomy is a valid procedure for the determination of the role of the kidney in the excretion of anticonvulsant drugs, this procedure should result in almost complete inability of the animal to inactivate or excrete barbital sodium. Hence, the ED50s for this drug after complete absorption should be practically constant with time. Conversely, nephrectomy should have no significant effect on the ED50s for pentobarbital sodium. Finally, carbon tetrachloride-induced liver damage should not alter significantly renal inulin clearance or the rate of barbital sodium excretion. Since measurement of inulin clearance and barbital sodium excretion reflect only changes in glomerular function, alterations in renal tubular function will not be revealed.

The results obtained will be evaluated in the discussion of each subsection on the basis of the working hypothesis. Any significant deviation from the hypothesis will be considered in the general discussion.

III. GENERAL PROCEDURES

Adult male albino rats of the Sprague-Dawley strain were used as experimental animals. They were maintained on Purina Laboratory Chow and allowed free access to food and water except during the actual test period. New animals were given one or two days to adjust to the laboratory environment and then given a series of three preliminary supramaximal shocks; the individual shocks were given every other day.

Maximal electroshock seizures (MES) were induced in each rat by 150 milliamperes of 60-cycle alternating current delivered for 0.2 second via corneal electrodes (Spiegel, 1937). All shocks were delivered by means of an apparatus described by Woodbury and Davenport (1952). Briefly, the procedure was as follows: A 1 per cent solution of butacaine sulfate was instilled into each conjunctival sac. The electrodes were placed in contact with the cornea and the stimulus was administered. The durations of hindleg-tonic flexion, hindleg-tonic extension, terminal generalized clonus, and total seizure were measured to the nearest half second. Duration of hindleg-tonic flexion was measured from the time of application of the stimulus to the time of onset of hindleg-tonic extension; duration of hindleg-tonic extension was measured from the time of onset of hindleg-tonic extension to the time of onset of terminal generalized clonus; duration of terminal generalized clonus was measured from the time of onset of the first clonic movement of any limb to the return of rapid respiration and/or skeletal muscle relaxation; total seizure was taken as the sum of these three components. Mean durations of the seizure

components and total seizure, with their respective standard deviations, were then calculated.

Groups of 16, 9, and 12 rats, respectively, were used for the studies of the effect of carbon tetrachloride-induced liver damage, hepatectomy, and nephrectomy on MES pattern. A MES was elicited in each animal every other day for six days. On the eighth day a fourth MES was elicited and the durations of the various components of the seizure were measured and recorded. This seizure was used as the control. Eight to 12 hours after the fourth shock, the first group, composed of 16 rats, was given carbon tetrachloride subcutaneously. Forty-eight hours later a MES was induced in each animal, and the durations of the various components of the seizure were measured and recorded. On the tenth day the second group, composed of nine rats, was hepatectomized; and the third group, composed of 12 rats, was nephrectomized. Twelve hours later a MES was induced in each animal, and the durations of the various components were measured and recorded. In all cases the mean durations of the various seizure components of normal animals were compared with those of the experimental animals; the difference was tested for statistical significance. In addition, groups of ten rats each were employed to study the effect of carbon tetrachloride-induced liver damage on MES pattern 3, 6, 12, 18, 24, and 36 hours after carbon tetrachloride administration; and two groups of four and nine rats were used to study the effect of nephrectomy on MES pattern 6 and 24 hours, respectively, after surgery.

The anticonvulsant activity of barbital sodium and pentobarbital sodium was estimated by the MES test (Toman et al., 1946; Swinyard, 1949)

in normal, carbon tetrachloride-treated, hepatectomized, and nephrectomized rats. Both drugs were administered orally as a 1 per cent (w/v) aqueous solution. The drugs were administered at a time which would permit the MES test to be conducted 48 hours after the administration of carbon tetrachloride, 12 hours after hepatectomy, or 12 hours after nephrectomy. Forty-eight hours after the administration of carbon tetrachloride was selected as the time for the test of liver-damaged animals because histological studies of liver sections demonstrate moderate to severe liver damage is present at this time (Swinyard et al., 1952a). Twelve hours after surgery was selected as the time for the test of hepatectomized and nephrectomized rats because this time interval was sufficient for the animals to recover from the anesthesia. Furthermore, this uniform period of time minimized individual differences in blood glucose levels in hepatectomized rats and in accumulated metabolic products in nephrectomized rats.

The dose of each drug which prevented the hindleg-tonic extensor component of maximal seizures in 50 per cent of animals tested (ED50) was determined as follows: Groups of four to ten animals were given various doses of the drug and subjected to the MES test at a previously selected time after drug administration. This procedure was repeated with different groups of animals until at least three dose levels were established between the limits of no protection and complete protection. The data obtained were plotted on logarithmic probability paper and a regression line visually fitted to the plotted points. The ED50 was determined from the plot of the data and 95 per cent fiducial limits were

calculated by the method of Litchfield and Wilcoxon (1949). This procedure was repeated with different groups of normal, carbon tetrachloride-treated, hepatectomized, and nephrectomized rats until ED50s and 95 per cent fiducial limits were determined 4, 8, and 12 hours after the administration of barbital sodium, and 1, 3, and 6 hours after the administration of pentobarbital sodium.

IV. THE EFFECTS OF CHEMICAL INJURY OF THE LIVER, PARTIAL HEPATECTOMY, AND NEPHRECTOMY ON MAXIMAL ELECTROSHOCK SEIZURE PATTERN AND ANTICONVULSANT ACTIVITY OF BARBITAL SODIUM AND PENTOBARBITAL SODIUM IN RATS

A. EFFECTS OF CARBON TETRACHLORIDE-INDUCED LIVER DAMAGE

1. Introduction

Several groups of workers (Pratt, 1933; Koppanyi et al., 1936; Richards et al., 1946; Moore et al., 1951; and others) have used carbon tetrachloride-induced liver damage as a biological procedure for the determination of the role of the liver in the fate and excretion of certain convulsant and anticonvulsant drugs. Because of the lack of agreement on the role of the liver in the fate and excretion of anticonvulsant agents, Swinyard and co-workers (1952) initiated a systematic study of the effects of carbon tetrachloride-induced liver damage on the activity of what then constituted the major clinically useful antiepileptic drugs. Their investigations indicated that the ED50s of mesantoin, phethenylate, diphenylhydantoin, trimethadione, paramethadione, and phenacemide, determined by the maximal electroshock seizure (MES) test, were significantly lower in liver-damaged animals than in normal animals (Swinyard et al., 1952a,b; Weaver et al., 1952). In 1954, this same group of workers investigated the effect of carbon tetrachloride-induced liver damage on the anticonvulsant activity of phenobarbital sodium and primidone. They found that the ED50s of these two drugs in liver-damaged animals were also lower than those determined in normal animals (Swinyard et al., 1954). Thus, it

appeared that in each instance the liver played an important role in the fate and excretion of the anticonvulsant drugs examined. Although this fact may be true, it was considered possible that either the liver damage or the carbon tetrachloride employed to induce the damage might alter the sensitivity of the central nervous system and hence the ED50s of the drugs tested. Therefore, it was thought important to test the effect of carbon tetrachloride-induced liver damage on MES pattern and on anticonvulsant activity of barbital sodium and pentobarbital sodium, two drugs whose fate and excretion are well known. The results obtained are presented in this section.

2. Methods

Carbon tetrachloride-induced liver damage was produced by a single subcutaneous injection of 2 ml./kgm. of a 50 per cent (w/v) solution of carbon tetrachloride in peanut oil. This injection was made into a loose fold of skin dorsomedially over the neck or back; shoulder and pelvic girdle areas were avoided.

3. Results

The results of these experiments are listed in tables 1, 2, and 3.

The lack of effect of carbon tetrachloride on the maximal electroshock seizure (MES) pattern is shown in table 1. It is evident that this procedure had no significant effect either on the duration of the various seizure components or on the total duration of the seizure 48 hours after carbon tetrachloride injection (flexion, $p > 0.05$; extension, $p > 0.5$; clonus, $p > 0.5$; and total duration, $p > 0.1$). On the other hand, one out of each group of ten rats was protected (i.e., exhibited no tonic-

TABLE 1

Maximal Electroshock Seizure Pattern

in Normal and Liver-Damaged Rats[1]

Treatment	No. of Animals	Mean Duration of Seizure Components, Seconds[2]			
		Tonic Hindleg		Clonus	Total Duration
		Flexion	Extension		
Normal	20	3.0 ± 0.10	7.1 ± 0.16	4.8 ± 0.14	14.9 ± 0.51
Liver Damage	18	3.3 ± 0.10	7.2 ± 0.17	4.8 ± 0.39	15.3 ± 0.41

[1]Determined 48 hours after subcutaneous administration of carbon tetrachloride.

[2] ±Standard deviation of the mean.

TABLE 2

The ED50s of Barbital Sodium Determined At Various Time Intervals After Drug Administration in Normal and Liver-Damaged Rats[1]

Time in Hours	Barbital Sodium, ED50[2] mgm./kgm.	
	Normal Rats	Liver-Damaged Rats
4	77.0 (66.4 - 89.3)	70.0 (58.8 - 83.3)
8	102.0 (83.3 - 130.0)	97.0 (79.2 - 118.8)
12	166.0 (138.3 - 199.2)	162.0 (135.5 - 200.0)

[1]Determined 48 hours after subcutaneous administration of carbon tetrachloride.

[2]Figures in parentheses represent 95 per cent fiducial limits.

TABLE 3

The ED50s of Pentobarbital Sodium Determined at Various Time Intervals After Drug Administration in Normal and Liver-Damaged Rats[1]

Time in Hours	Pentobarbital Sodium, ED50[2] mgm./kgm.	
	Normal Rats	Liver-Damaged Rats
1	16.8 (14.4 - 19.7)	6.5 (5.7 - 7.4)
3	42.5 (38.6 - 46.8)	10.0 (8.9 - 11.2)
6	60.0 (51.7 - 69.6)	28.0 (22.4 - 35.0)

[1]Determined 48 hours after subcutaneous administration of carbon tetrachloride.

[2]Figures in parentheses represent 95 per cent fiducial limits.

extensor component) 3, 6, and 12 hours after carbon tetrachloride administration, whereas no protection was observed at 8, 24, 36, or 48 hours.

The anticonvulsant activity of barbital sodium as measured in normal and liver-damaged rats is shown in table 2. It may be seen from the table that the ED50s for both normal and liver-damaged animals are similar; indeed, they are almost identical.

The anticonvulsant activity of pentobarbital sodium as measured in normal and liver-damaged rats is shown in table 3. It may be seen from the table that the ED50s of liver-damaged animals are significantly lower at all time intervals than the ED50s of normal animals.

4. Discussion

The data concerned with the effect of carbon tetrachloride administration on the maximal electroshock seizure (MES) pattern indicates an early anticonvulsant effect of this agent. However, since all definitive tests were conducted at the 48-hour period, and since no significant pattern modification was detected beyond 24 hours after carbon tetrachloride injection, it must be concluded that neither carbon tetrachloride administration nor the liver damage produced by it significantly alters the ability of the central nervous system to respond normally to supramaximal electrical stimulation after this period of time.

In order to see how well the data concerned with the anticonvulsant action of barbital sodium and pentobarbital sodium, determined at various time intervals in normal and liver-damaged rats, fit the hypothesis formulated in section II, the ED50s presented in tables 2 and 3 were graphically plotted as shown in figures 2 and 3, and smooth curves were drawn through

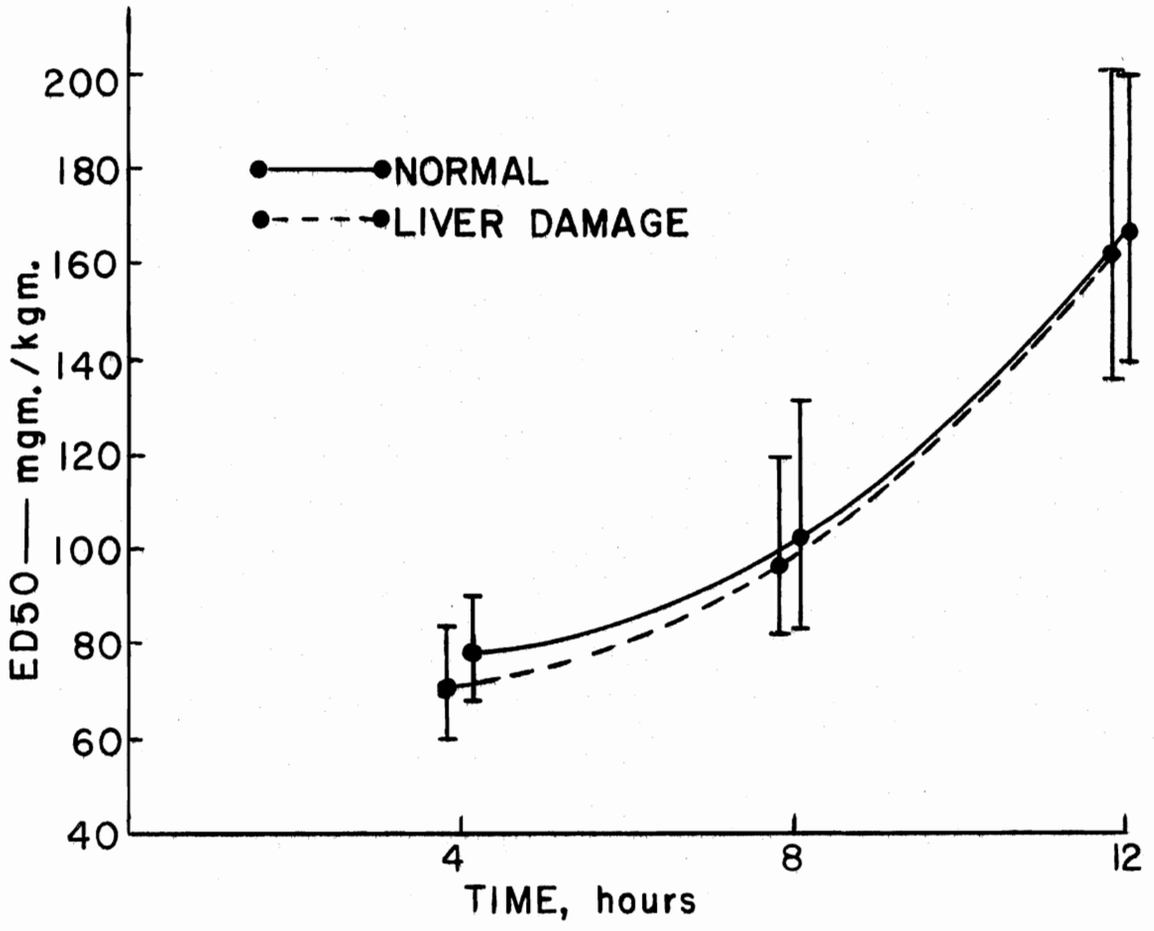

Figure 2. The ED50s of Barbital Sodium Determined at Various Time Intervals After Drug Administration in Normal and Liver-Damaged Rats.

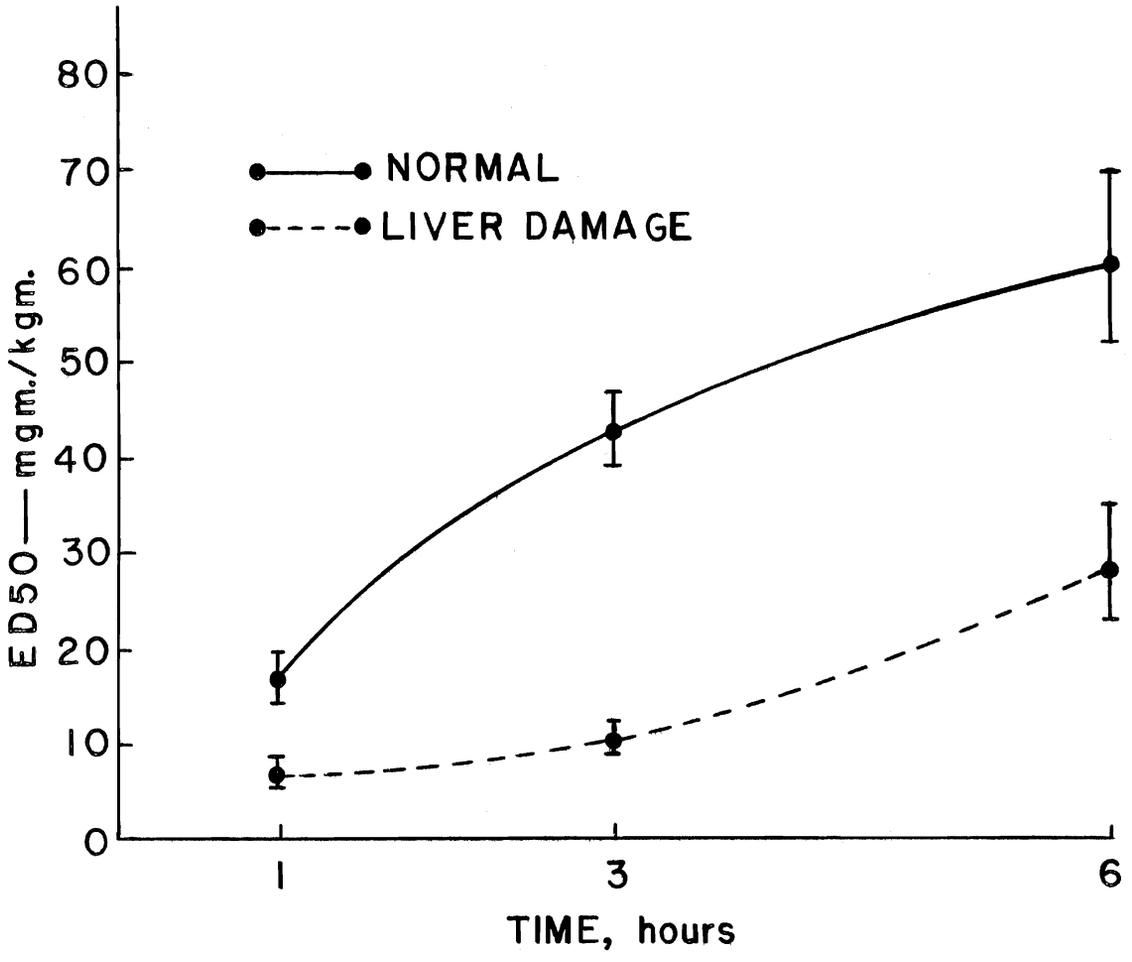

Figure 3. The ED50s of Pentobarbital Sodium Determined at Various Time Intervals After Drug Administration in Normal and Liver-Damaged Rats.

the points. As shown in figure 2, the two curves for barbital sodium are virtually identical in normal and liver-damaged rats. In contrast, it may be seen in figure 3 that the curve connecting the ED50s for pentobarbital sodium determined in liver-damaged rats is displaced toward the abscissa at all time intervals and, in addition, appears to be divergent to the normal curve. In general, these plots of the data are in agreement with the hypothesis and suggest that carbon tetrachloride-induced liver damage is a valid procedure for the determination of the role of this organ in the fate and excretion of anticonvulsant drugs.

B. EFFECTS OF HEPATECTOMY

1. Introduction

As mentioned in the introduction to the previous section, chemical agents used to induce liver damage may adversely affect other organs or systems in addition to the liver. Thus, the use of a hepatotoxic agent may "color" experimental results in such a way as to give false information about the true role of the liver in the fate and excretion of drugs tested. Therefore, it was thought important to study in rats the effects of partial hepatectomy in order to observe the effect of the physiological alterations produced by the surgical removal of liver tissue on the maximal electroshock seizure (MES) pattern and the anticonvulsant activity of barbital sodium and pentobarbital sodium.

2. Methods

A rat was lightly anesthetized with ether, fastened ventral side up on the operating table, and hepatectomy was performed by the method described by Timiras (1955). Anesthesia was maintained by placing a small beaker containing an ether-saturated pledget of cotton over the nose of the animal. The rat was shaved, the xiphoid process was palpated, and a midline incision made through the skin approximately one-half to three-quarters of an inch distal to the tip of the xiphoid. The incision was continued up to and just over this distal tip of the process. The peritoneal cavity was opened with a similar incision. The tip of the exposed xiphoid process was grasped with a rat-toothed forceps, and the rounded one-quarter inch of this cartilagenous structure was cut off with a pair of heavy scissors. This procedure provided an opening sufficiently large

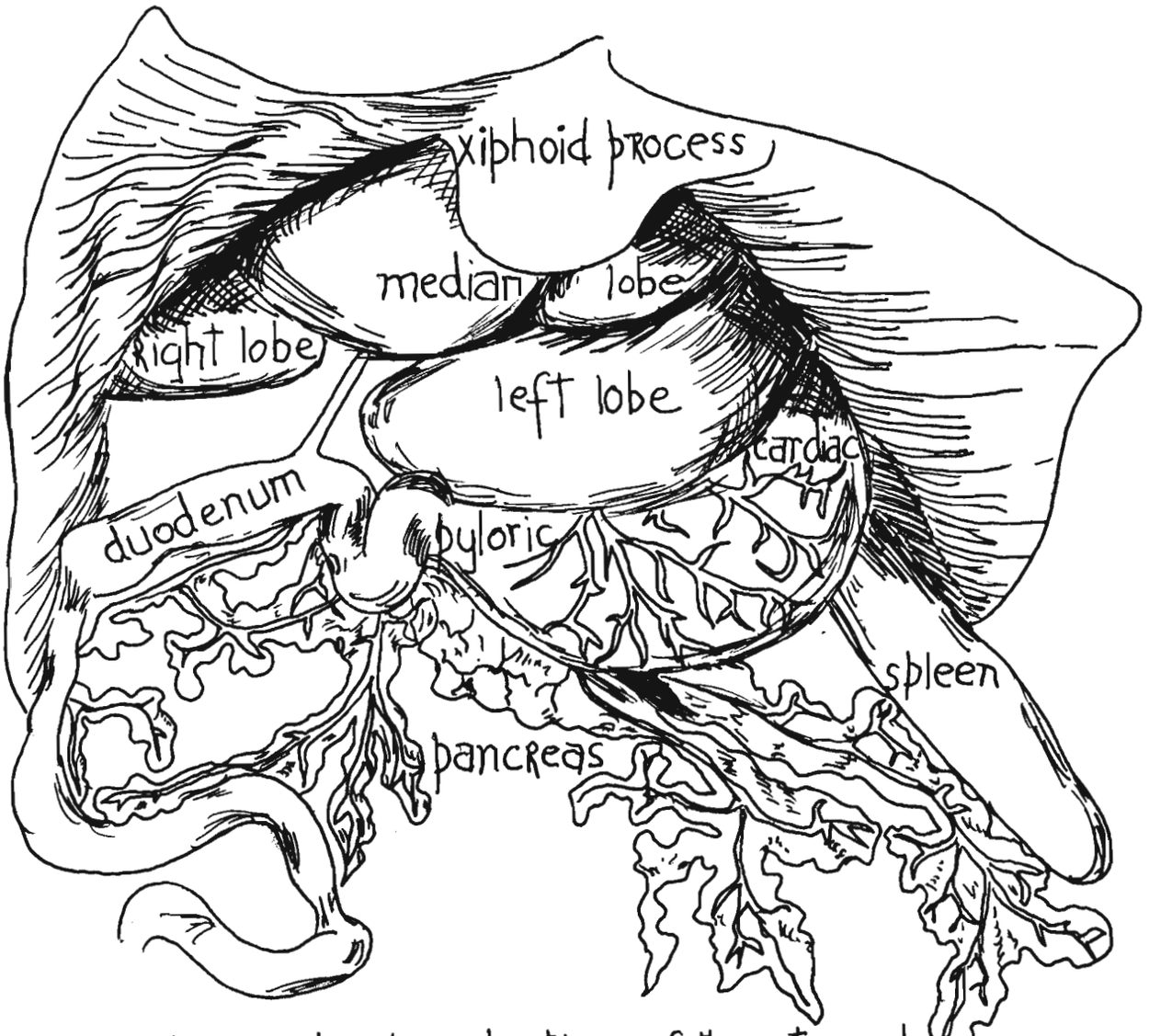

Figure 4. Cardiac and pyloric portions of the stomach, the pancreas, and liver. Camera lucida.*

*E. C. Greene. Anatomy of the Rat. New York, Hafner Publishing Co. 1955. p. 101.

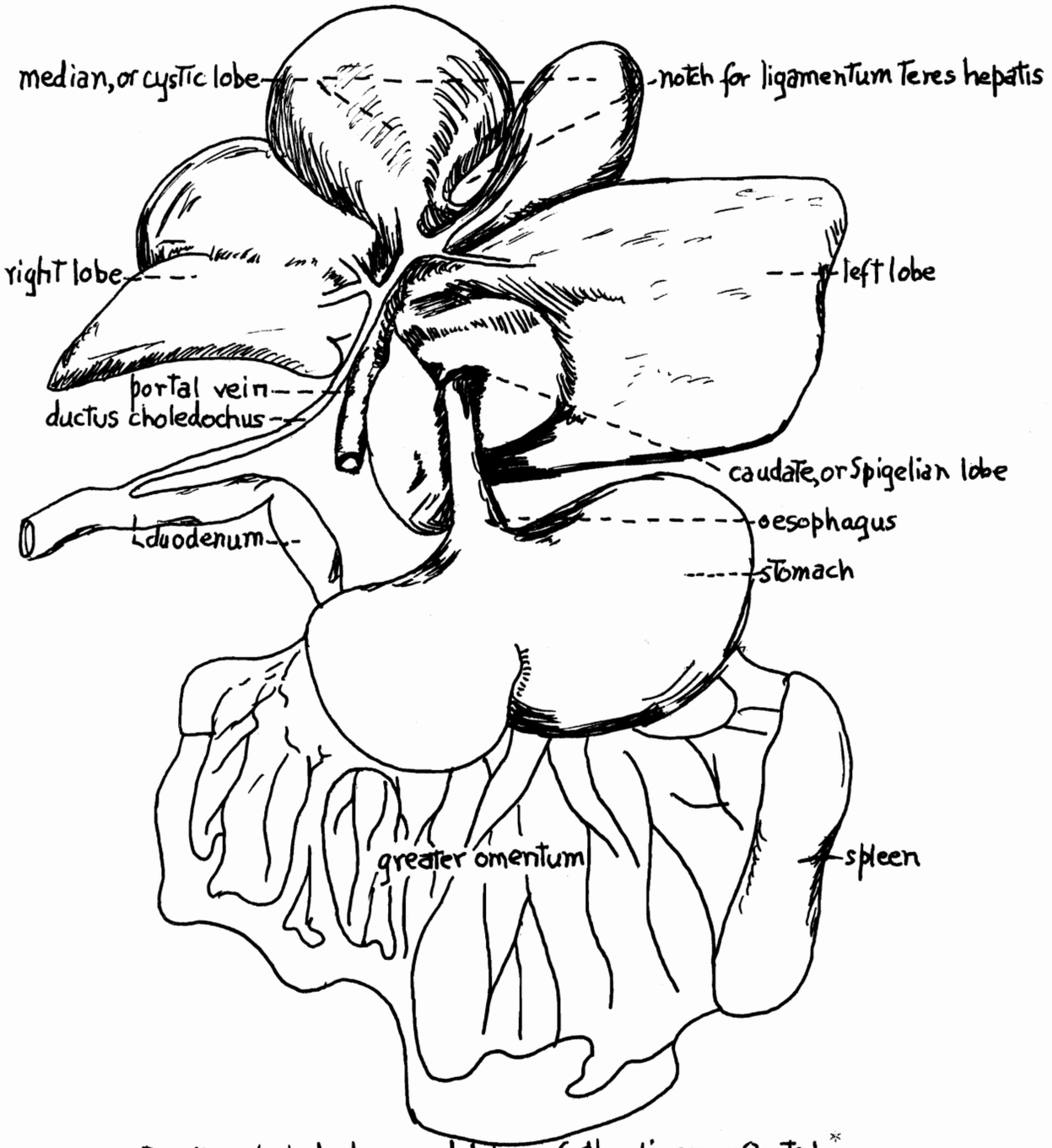

Figure 5. Ductus choledochus and lobes of the liver, reflected.*

*E. C. Greene. Anatomy of the Rat. New York, Hafner Publishing Co. 1955. p. 101.

to withdraw the liver. The liver presents four parts (figures 4 and 5): A median or cystic lobe, bearing a deep fissure for the hepatic ligament; a right lateral lobe, partly divided into anterior and posterior lobules; a large left lateral lobe; and a small caudate lobe which lies deep on the left side and fits around the esophagus. The median and left lateral lobes were then grasped with the left hand and drawn upward through the incision. The deep-lying caudate lobe then became visible. With cautious probing, this lobe was freed from the esophagus. It was then drawn upward and held with the median and left lateral lobes. A mass ligature of nylon thread was passed around these three lobes as far proximal as possible and secured tightly. All of the liver distal to the suture was then surgically ablated. The liver removed represents approximately 73 per cent by weight of the total rat liver. The peritoneal cavity was then closed using nylon thread and an interrupted suture, and the skin was closed with 11 mm. Michel wound clips. An extremely good closure was found to be imperative; otherwise, the animal would reopen the incision in a matter of a few hours.

3. Results

The results of these experiments are listed in tables 4, 5, and 6.

The effect of hepatectomy on the maximal electroshock seizure (MES) pattern is shown in table 4. It is evident that this procedure had no significant effect on the duration of the flexor or extensor component or on the total duration of the seizure at the time of test, i.e., 12 hours after surgery (flexion, $p > 0.1$; extension, $p > 0.5$; and total duration, $p > 0.05$). The duration of the clonic component was significantly different from normal at the 5 per cent level ($p = 0.05$).

TABLE 4

Maximal Electroshock Seizure Pattern

in Normal and Hepatectomized Rats[1]

Treatment	No. of Animals	Mean Duration of Seizure Components, Seconds[2]			
		Tonic Hindleg		Clonus	Total Duration
		Flexion	Extension		
Normal	9	3.5 ± 0.14	6.7 ± 0.17	4.8 ± 0.22	15.0 ± 0.24
Hepatectomy	9	3.8 ± 0.22	6.5 ± 0.37	5.8 ± 0.40	16.1 ± 0.55

[1]Determined 12 hours after hepatectomy.

[2] ± Standard deviation of the mean.

TABLE 5

The ED50s of Barbital Sodium Determined at Various Time Intervals After Drug Administration in Normal and Hepatectomized Rats[1]

Time in Hours	Barbital Sodium, ED50[2] mgm./kgm.	
	Normal Rats	Hepatectomized Rats
4	71.0 (58.7 - 85.9)	82.0 (69.5 - 95.6)
8	141.0 (111.9 - 177.7)	128.0 (107.6 - 152.3)
12	190.0 (162.4 - 222.3)	158.0 (121.5 - 205.4)

[1]Hepatectomy performed 12 hours previous to drug administration.

[2]Figures in parentheses represent 95 per cent fiducial limits.

TABLE 6

The ED50s of Pentobarbital Sodium Determined at Various Time Intervals After Drug Administration in Normal and Hepatectomized Rats[1]

Time in Hours	Pentobarbital Sodium, ED50[2] mgm./kgm.	
	Normal Rats	Hepatectomized Rats
1	17.0 (12.8 - 22.6)	9.8 (7.5 - 12.8)
3	32.5 (29.0 - 36.4)	15.6 (13.9 - 17.6)
6	47.0 (39.5 - 55.9)	18.2 (15.2 - 21.8)

[1]Hepatectomy performed 12 hours previous to drug administration.

[2]Figures in parentheses represent 95 per cent fiducial limits.

The anticonvulsant activity of barbital sodium determined at various time intervals after drug administration in normal and hepatectomized rats is shown in table 5. It is apparent from the table that the ED50s for this drug are essentially the same whether determined in normal or hepatectomized animals.

The anticonvulsant activity of pentobarbital sodium determined at various time intervals after drug administration in normal and hepatectomized rats is shown in table 6. It may be seen from the table that the ED50s determined in hepatectomized animals are significantly lower than the ED50s determined in normal animals at all time intervals after drug administration.

4. Discussion

The data indicate that hepatectomy modified somewhat the pattern of maximal electroshock seizures (MES) determined 12 hours after surgery. This is characterized by an increase in the duration of the clonic component which is significant at the 5 per cent level (see table 4). In addition, the slight decrease in the duration of hindleg-tonic flexion and slight increase in duration of hindleg-tonic extension and of total seizure are in the direction expected if the operative procedure did indeed decrease the sensitivity of the central nervous system. These observations suggest that hepatectomy alters the response of the central nervous system to supramaximal electrical stimulation at the twelfth hour after surgery.

In order to see how well the data concerned with the anticonvulsant action of barbital sodium and pentobarbital sodium, determined in normal

and hepatectomized rats, fit the hypothesis formulated in section II, the ED50s presented in tables 5 and 6 were graphically plotted as shown in figures 6 and 7; and smooth curves were drawn through the points. As shown in figure 6, the two curves for barbital sodium are similar in normal and hepatectomized rats. In contrast, it may be seen in figure 7 that the curve connecting the ED50s for pentobarbital sodium in hepatectomized rats is displaced significantly toward the abscissa and, in addition, appears to be divergent to the normal curve. It is concluded that, in general, these data are in agreement with the working hypothesis.

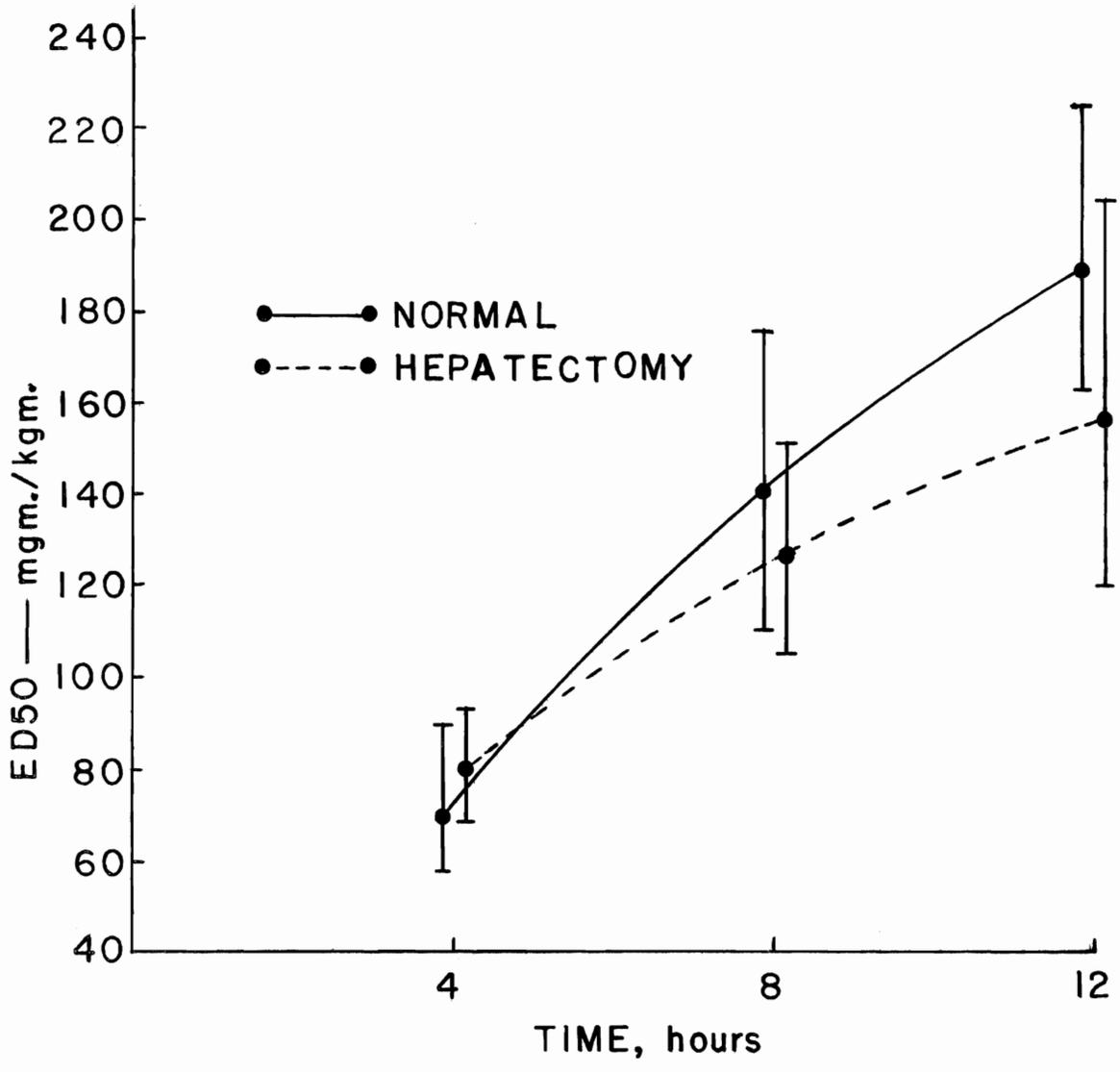

Figure 6. The ED50s of Barbital Sodium Determined at Various Time Intervals After Drug Administration in Normal and Hepatectomized Rats.

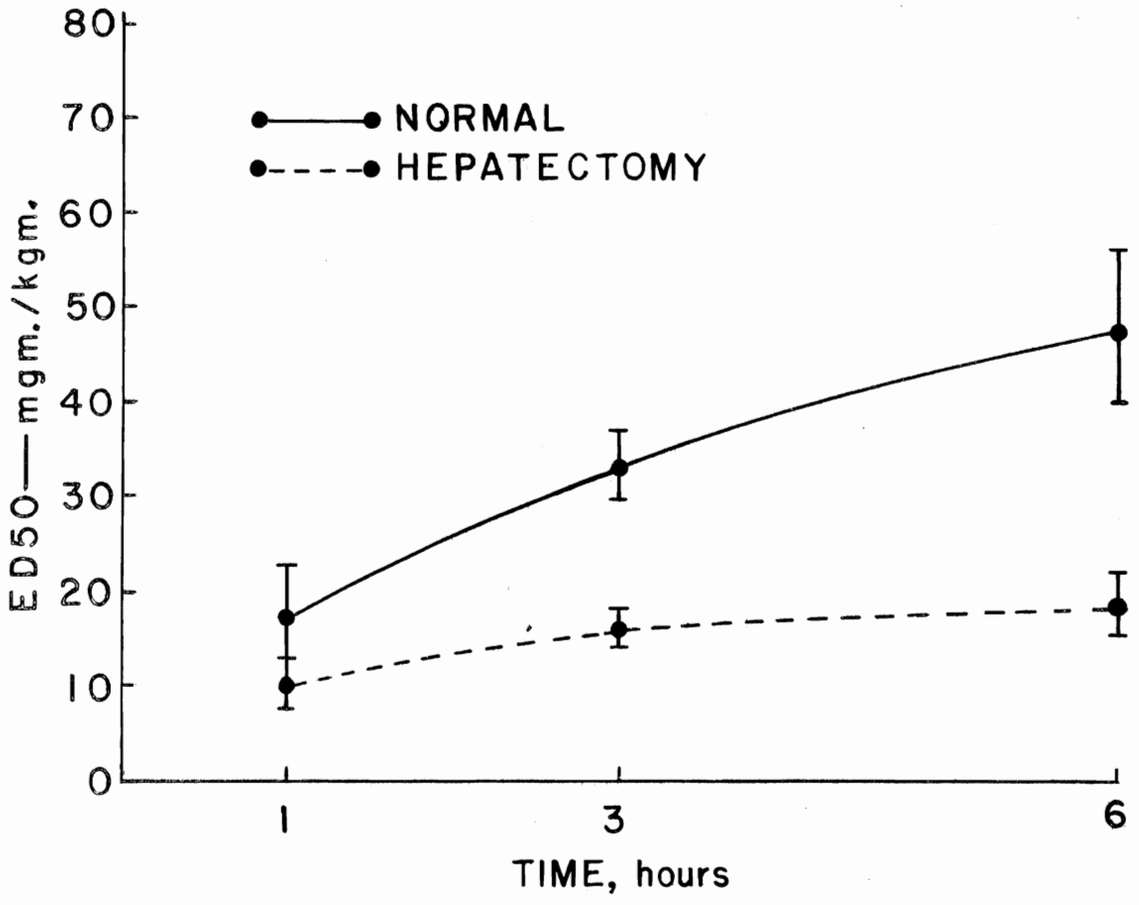

Figure 7. The ED50s of Pentobarbital Sodium Determined at Various Time Intervals After Drug Administration in Normal and Hepatectomized Rats.

C. EFFECTS OF NEPHRECTOMY

1. Introduction

Despite the fact that nephrectomy has been used to study the role of the kidney in the fate and excretion of anticonvulsant drugs (Richards et al., 1946; Moore et al., 1951; Swinyard et al., 1952a,b; Weaver et al., 1952; Swinyard et al., 1954), the validity of this biological procedure for the determination of the role of the kidney in the fate and excretion of anticonvulsant drugs has not been unequivocally established.

Considerable work has been done in the pharmacology laboratories of the University of Utah on the effects of nephrectomy on the pattern of maximal electroshock seizures (MES), on the threshold for minimal electroshock seizures, and on the activity of anticonvulsant drugs. Swinyard and co-workers (1952a) have shown that bilateral nephrectomy had no significant effect on the MES pattern 12 and 18 hours after surgery. Schiffman and co-workers (1955b) and Tedeschi (1955), in similar studies, confirmed these observations. With regard to electroshock seizure threshold, Woodbury and co-workers (1950), and Woodbury (1954, 1957) observed a 3 and 19 per cent increase in threshold 7 and 24 hours, respectively, after nephrectomy. With regard to anticonvulsant activity, Swinyard and co-workers (1952a,b; 1954) found that the ED50s for phethenylate, paramethadione and primidone were significantly lower in nephrectomized animals than in normal animals, whereas this biological alteration had no significant effect on the ED50s of diphenylhydantoin, Mesantoin, trimethadione, and phenobarbital. Weaver and co-workers (1952) noted that nephrectomy had no effect on the fate and excretion of phenacemide.

Except for the effect of nephrectomy on electroshock seizure threshold, these results suggest that nephrectomy is a valid biological procedure for the study of the fate and excretion of anticonvulsant drugs. Nevertheless, the fact that seizure threshold is increased after nephrectomy suggests that further study of this problem is warranted. Therefore, the effects of nephrectomy on the MES pattern and on the anticonvulsant activity of barbital sodium and pentobarbital sodium were determined.

2. Methods

Bilateral nephrectomy was performed by the retroperitoneal approach. The rat was first lightly anesthetized with ether and fastened ventral side down on the operating table. Anesthesia was maintained by placing a small beaker containing an ether-saturated pledget of cotton over the nose of the animal. The back of the animal was shaved, and a medial incision approximately 1 inch long was made in the skin over the lower back. The exposed dorsal lumbar muscles on both sides were incised. The kidneys were palpated through the abdominal wall and forced through the incisions in the muscle coat. A mass ligature of nylon thread was passed around the underside of the kidney as far proximal as possible and tightly secured. The kidney distal to the suture was removed. The muscle wall was closed with nylon thread and the skin incision with Michel wound clips.

3. Results

The results of these experiments are listed in tables 7, 8, and 9.

The lack of effect of nephrectomy on the maximal electroshock seizure (MES) pattern is shown in table 7. It is evident that this procedure had no significant effect either on the durations of the various seizure

TABLE 7

Maximal Electroshock Seizure Pattern

in Normal and Nephrectomized Rats[1]

Treatment	No. of Animals	Mean Duration of Seizure Components, Seconds[2]			
		Tonic Hindleg		Clonus	Total Duration
		Flexion	Extension		
Normal	12	3.0 ± 0.12	7.9 ± 0.32	4.9 ± 0.26	16.8 ± 0.33
Nephrectomy	12	3.5 ± 0.24	7.4 ± 0.62	4.4 ± 0.63	16.7 ± 0.40

[1]Determined 12 hours after nephrectomy.

[2] ±Standard deviation of the mean.

TABLE 8

The ED50s of Barbital Sodium Determined at Various Time Intervals After Drug Administration in Normal and Nephrectomized Rats[1]

Time in Hours	Barbital Sodium, ED50[2] mgm./kgm.	
	Normal Rats	Nephrectomized Rats
4	77.0 (66.4 - 89.3)	96.0 (73.8 - 124.8)
8	102.0 (83.3 - 130.0)	92.0 (78.6 - 107.5)
12	166.0 (138.3 - 199.2)	89.0 (82.6 - 95.9)

[1]Determined 12 hours after nephrectomy.

[2]Figures in parentheses represent 95 per cent fiducial limits.

TABLE 9

The ED50s of Pentobarbital Sodium Determined at Various Time Intervals After Drug Administration in Normal and Nephrectomized Rats[1]

Time in Hours	Pentobarbital Sodium, ED50[2] mgm./kgm.	
	Normal Rats	Nephrectomized Rats
1	16.8 (14.4 - 19.7)	17.9 (15.2 - 21.1)
3	42.5 (38.6 - 46.8)	41.5 (37.4 - 46.1)
6	60.0 (51.7 - 69.6)	59.0 (54.4 - 64.0)

[1]Determined 12 hours after nephrectomy.

[2]Figures in parentheses represent 95 per cent fiducial limits.

components or on the total duration of the seizure at the time of test, i.e., 12 hours after surgery (flexion, $p > 0.05$; extension, $p > 0.5$; clonus, $p > 0.1$; and total duration, $p > 0.5$). Other groups of rats, shocked 6 and 24 hours after nephrectomy, also showed no significant alteration in MES pattern.

The anticonvulsant activity of barbital sodium as measured in normal and nephrectomized rats is shown in table 8. It may be seen from the table that the ED50s determined in nephrectomized rats are similar at all time intervals. In contrast, the quantity of drug required to protect 50 per cent of normal animals increases progressively with time; by the twelfth hour the ED50 is more than twice the ED50 determined at the fourth hour.

The anticonvulsant activity of pentobarbital sodium as measured in normal and nephrectomized rats is shown in table 9 from which it may be seen that the ED50s at the various time intervals are almost identical in the two groups of animals. Although the ED50s for pentobarbital sodium 6 hours after drug administration are approximately 3.5 fold greater than those determined at 1 hour, there is no significant difference in the ED50s determined in normal and nephrectomized rats at any particular time interval.

4. Discussion

The data concerned with the effect of nephrectomy on the maximal electroshock seizure (MES) pattern clearly show that there is no significant change in any components of the pattern even as long as 24 hours after surgery. Since all definitive tests were conducted 12 hours after the operation, it must be concluded that neither nephrectomy nor the accumulation

of metabolic products as a result of this procedure significantly alter the ability of the central nervous system to respond normally to supramaximal electrical stimulation at this time interval.

In order to see how well the data concerned with the anticonvulsant action of barbital sodium and pentobarbital sodium fit the hypothesis formulated in section II, the ED50s presented in tables 8 and 9 were graphically plotted in figures 8 and 9 and smooth curves were drawn through the points. As shown in figure 8, the curve connecting the ED50s for barbital sodium is essentially parallel to the abscissa since the ED50s for this drug are almost identical at all three time intervals. On the other hand, the normal curve is markedly divergent to both the abscissa and the curve for nephrectomized rats. In contrast, it may be seen in figure 9 that the two curves for pentobarbital sodium are virtually identical in normal and nephrectomized rats. In general, these plots of the data are in agreement with the hypothesis and suggest that nephrectomy is a valid procedure for the determination of the role of this organ in the fate and excretion of anticonvulsant drugs.

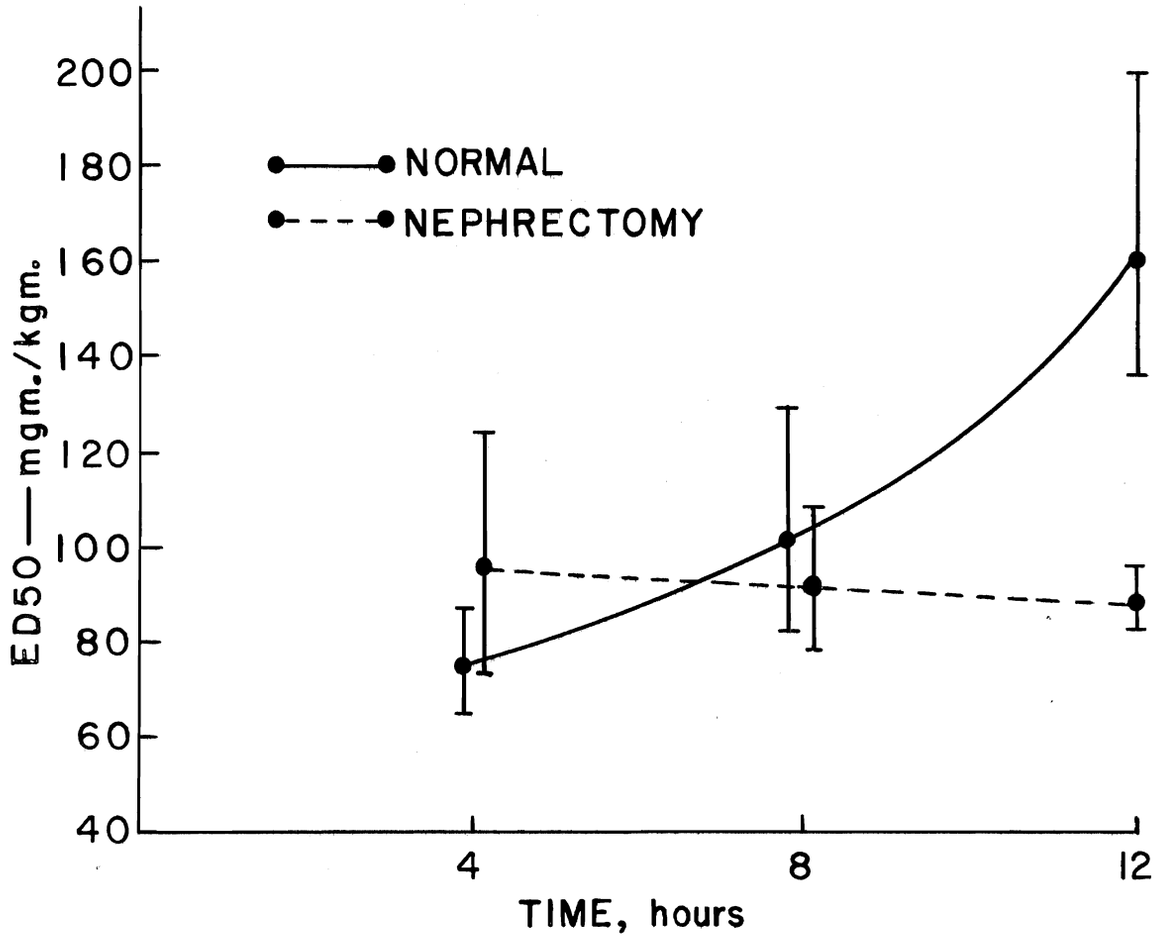

Figure 8. The ED50s of Barbital Sodium Determined at Various Time Intervals After Drug Administration in Normal and Nephrectomized Rats.

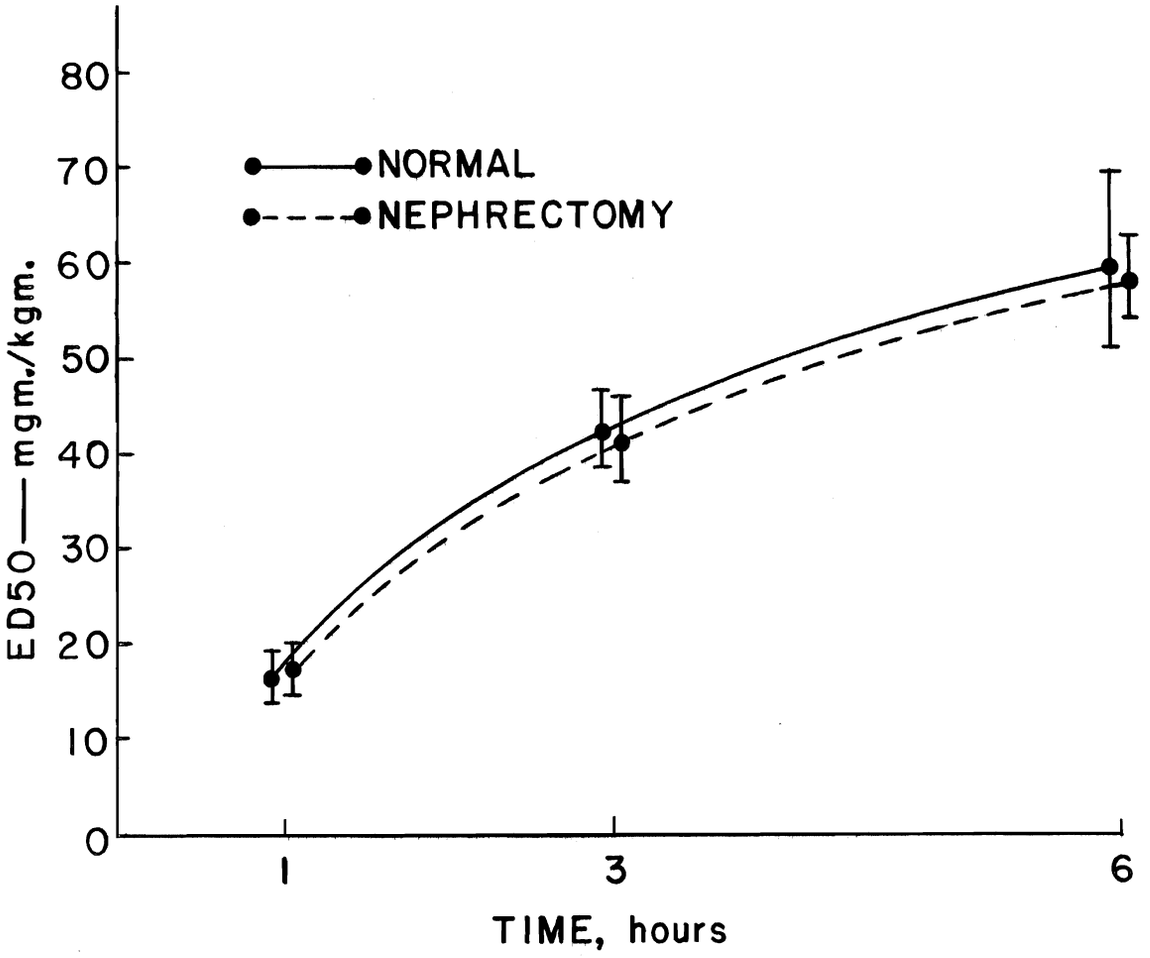

Figure 9. The ED50s of Pentobarbital Sodium Determined at Various Time Intervals After Drug Administration in Normal and Nephrectomized Rats.

V. THE EFFECTS OF CARBON TETRACHLORIDE ADMINISTRATION ON RENAL FUNCTION IN THE RAT

A. EFFECTS ON INULIN CLEARANCE

1. Introduction

Since renal damage has been reported to occur after the administration of carbon tetrachloride to laboratory animals (Meyer and Pessoa, 1923; Smyth et al., 1936; Opie, 1950; Ungar, 1951; Jennings and Kearns, 1953), it was thought that glomerular filtration rate and/or tubular function might be markedly depressed in rats treated with this agent. Either a decreased renal filtration rate or a depressed tubular function could markedly alter the quantity of drug excreted by the kidney. Therefore, it was felt that the results obtained from a renal function test might reveal the effects of carbon tetrachloride on the renal excretion of anticonvulsant drugs. Accordingly, studies on the renal clearance of inulin were conducted in normal and liver-damaged rats. However, since this procedure provides only an estimate of glomerular filtration rate (Smith, 1951), data concerned with the effect of carbon tetrachloride administration on renal tubular function were not obtained.

2. Methods

Twenty adult female albino rats of the Sprague-Dawley strain were divided into two groups of ten animals each. One group served as a control, whereas the other group was given carbon tetrachloride subcutaneously (see section IV, page 17). Both groups of animals were housed in a constant temperature room maintained at 26° C. and were brought to a uniform state

of hydration by the oral administration of warm tap water (25 ml./kgm.) 12 to 18 hours prior to the actual experiment. Food was withdrawn at this time, but water was allowed ad libitum until the experiment started. All experiments were conducted in a constant temperature room also maintained at 26° C. At the time of the experiment, 47 hours after carbon tetrachloride administration, a rat was lightly anesthetized with ether. A small polyethylene tube, held rigid by means of a wire stylette inside the tube and with numerous holes near the end which is to dwell inside the bladder, was passed through the urethra and into the bladder. The stylette was withdrawn and rubber cement applied externally around the urethra to hold the emerging tube in place. The animal was allowed to recover from the ether and was hydrated with 0.2 per cent sodium chloride solution (5 ml./100 Gm.) orally. The rat was then given heparin solution containing 10,000 U.S.P. heparin units per ml. (0.5 ml./animal) intraperitoneally, and 10 per cent inulin solution (1000 mgm./kgm.) intraperitoneally. The animal was placed in a holder of the type employed in the blood pressure method of Kersten and co-workers (1947); this device restrained the animal, but allowed the tail and the polyethylene tubing to be externalized. The tail of the rat was infiltrated around the base with dibucaine (Nupercaine) hydrochloride solution (1:500) in sufficient quantity to prevent pain perception as judged by the lack of vocalization or other overt response to tail pinching. This procedure was intended to prevent possible initiation of reflex vasoconstriction in response to blood sampling. One-fourth inch of the tip of the tail was removed and a silk ligature secured proximal to the cut end to prevent hemorrhage. Forty to 45 minutes after hydration with the sodium

chloride solution (i.e., 48 hours after carbon tetrachloride administration), the bladder was washed four to six times with 0.25 ml. volumes of distilled water. The urine excreted during the next 18 minutes was collected in a calibrated 12 ml. centrifuge tube. Again the bladder was washed four to six times with 0.25 ml. volumes of distilled water; these washings were added to the urine sample. Total collection time, therefore, was approximately 20 minutes. At the midpoint of the collection period the ligature on the tail was removed, the first few drops of blood were discarded, and approximately 0.4 ml. of blood was obtained in a length of 4 mm. glass capillary tubing. One end of the blood tube was then sealed with plasticine, and the tube was centrifuged at 3000 r.p.m. for 30 minutes. After centrifugation, the tube was scratched at the cell-plasma interface and broken. The plasma was taken up in a pipette, measured, and transferred to a 25 ml. Erlenmeyer flask for chemical analysis. All samples were analyzed by the method described on page 58.

3. Results

The glomerular filtration rate, as measured by renal inulin clearance, was found to be 0.358 ± 0.030 ml./100 cm.^{2}/minute in normal rats, and 0.252 ± 0.032 ml./100 cm.^{2}/minute in liver-damaged rats. Statistical analysis indicated that the filtration rate in liver-damaged rats was not significantly different from normal ($p > 0.2$).

4. Discussion

The trend toward a decreased glomerular filtration rate in carbon tetrachloride-treated rats, as indicated by the decrease in renal inulin clearance, is in agreement with the observations of Corcoran and co-workers

(1943) and Sirota (1949) in man. Although the decreased filtration rate in the carbon tetrachloride-treated rats was not statistically significant, it suggests that if a drug is excreted unchanged by the kidney and primarily filtered through the glomeruli a decreased glomerular filtration rate, however slight, might tend to keep blood levels of the drug higher for a longer period of time in kidney-damaged rats than in normal rats. Of course, the foregoing does not take into account any changes in tubular function which might also be present and which could affect tubular transport of drugs either secreted or reabsorbed by tubular cells. Thus, the approximate 30 per cent decrease in the filtration rate and the possibility that carbon tetrachloride might alter renal transport mechanisms appear to be of sufficient importance to warrant further investigation of the influence of this agent on the kidney despite the fact that, in general, the results obtained are in agreement with the working hypothesis formulated in section II. The results of studies designed to reveal the effects in rats of carbon tetrachloride administration on the rate at which barbital sodium is excreted by the kidney are presented in the next section.

B. EFFECTS ON BARBITAL SODIUM EXCRETION

1. Introduction

Because carbon tetrachloride tended to decrease glomerular filtration rate, as indicated in the previous section, it was thought that some insight into the extent of kidney damage might be revealed by a study of the effect of this agent on the renal excretion of a drug known to be eliminated by the kidney. Since barbital sodium has been reported to be filtered only by the glomeruli (Arakawa, 1935; Argy et al., 1936) and quantitatively excreted unchanged by the kidney (Maynert and van Dyke, 1949), this agent was selected for renal excretion studies in normal and liver-damaged rats. The results obtained are presented in this section.

2. Methods

Twenty adult male albino rats were given the ED50 of barbital sodium for normal rats (77 mgm./kgm.) orally as a 1 per cent (w/v) aqueous solution. The rats were then placed in metabolism cages in groups of four, and the urine excreted by each group during the ensuing 16-hour period was collected. Urine was collected in small-mouthed Erlenmeyer flasks to prevent excessive evaporation. Urine samples were taken for the periods of 0 to 4, 4 to 8, 8 to 12, and 12 to 16 hours, and were analyzed for barbital sodium according to the method described on page 60. Two weeks later these same animals were given carbon tetrachloride subcutaneously (see section IV, page 17) and 36 hours later were given the same dose of barbital sodium. The urine was collected as described above and samples were taken for the periods of 0 to 4, 4 to 8, 8 to 12, 12 to 16, and 16 to 18 hours and analyzed for barbital sodium content (see above).

The amount of barbital sodium excreted in normal animals was compared to the amount excreted in liver-damaged animals, and the difference was tested for statistical significance.

3. Results

The total urinary excretion of barbital sodium in normal and liver-damaged rats after the oral administration of 77 mgm./kgm. of the drug is given in table 10. It may be seen from the table that within the first 4 hours after drug administration there is no significant difference in the excretion of barbital sodium, whereas by the twelfth hour the excretion of barbital sodium is decreased significantly in the liver-damaged animals ($p < 0.01$). Thus, 54.06 mgm./kgm. of barbital sodium were excreted by liver-damaged rats in 12 hours, whereas a total of 73.05 mgm./kgm. was excreted in normal rats during the same time interval. However, by the sixteenth hour there was no significant difference in the amount of barbital sodium excreted by the two groups.

4. Discussion

The data presented indicate that rats treated with carbon tetrachloride take a longer time to excrete a given dose of barbital sodium than do normal rats. This evidence, in addition to the slight decrease in glomerular filtration rate as measured by inulin clearance, suggests that carbon tetrachloride administration decreases glomerular function. These findings are not in agreement with the hypothesis set forth in section II and will be considered in more detail in the general discussion.

TABLE 10

The Renal Excretion of Barbital Sodium

in Normal and Liver-Damaged Rats[1]

Time in Hours	Barbital Sodium Excreted[2] mgm./kgm.	
	Normal Rats	Liver-Damaged Rats
4	13.30 ± 4.26	11.74 ± 2.89
8	44.48 ± 4.01	30.99 ± 3.79
12	73.05 ± 3.33	54.06 ± 5.84
16	*	72.95 ± 9.83

[1]Carbon tetrachloride administered 36 hours previous to drug administration.

[2] ±Standard deviation of the mean.

*A value for the 16-hour period in normal rats was omitted because the amount of barbital sodium excreted during the 12 to 16-hour period was negligible.

VI. GENERAL DISCUSSION

The effects of carbon tetrachloride-induced liver damage, partial hepatectomy and bilateral nephrectomy on the maximal electroshock seizure (MES) pattern and on the effective doses fifty (ED50s) of barbital sodium and pentobarbital sodium are graphically summarized in table 11.

It may be seen from the table that, in general, the results obtained from the present study are in agreement with both the known fate and excretion of the two barbiturates employed and with the working hypothesis set forth in section II of this dissertation. However, it will be noted that there are certain discrepancies which do not fit either the fate and excretion pattern of barbital sodium and pentobarbital sodium or the formulated hypothesis. These differences will be given attention at this time.

With regard to carbon tetrachloride-induced liver damage, the data presented in section IV-A indicate that the ED50s for barbital sodium are remarkably similar in normal and liver-damaged rats. In contrast, the ED50s for pentobarbital sodium are markedly lower in carbon tetrachloride-treated rats than in normal rats. When the ED50s for pentobarbital sodium are plotted as shown in figure 3, page 23, the possibility that the two curves may approach parallelism and thereby be indicative of an increase in the sensitivity of the animal and/or a progressive accumulation of metabolic products which act additively with the effect of the drug has not been ruled out. Since there was no evidence of an increased sensitivity of the animal or of an additive effect when barbital sodium was tested in carbon tetrachloride-treated rats, it must be concluded that the decrease in the ED50s for pentobarbital sodium reflects the inability of liver-damaged

TABLE 11

Summary of the Effects of Carbon Tetrachloride-Induced Liver Damage, Partial Hepatectomy, and Nephrectomy on Maximal Electroshock Pattern and ED50s of Barbital Sodium and Pentobarbital Sodium

	Carbon Tetrachloride-Induced Liver Damage	Partial Hepatectomy	Nephrectomy
Pattern			
Flexion	↔	↔	↔
Extension	↔	↔	↔
Clonus	↔	↑	↔
Total Duration	↔	↔	↔
Barbital Sodium ED50 Against MES	↔	↔	↓
Pentobarbital Sodium ED50 Against MES	↓	↓	↔

↔ = no change ↓ = decrease ↑ = increase

rats to inactivate pentobarbital sodium and, hence, are in agreement with the formulated hypothesis.

In the case of partial hepatectomy, there are two problems which are worthy of consideration. Firstly, MES pattern was slightly modified in hepatectomized rats. This observation raises the question as to whether hypoglycemia or other metabolic alterations induced by removal of part of the liver were present. Studies designed to explore these possibilities were not conducted since partial hepatectomy was employed only to rule out the possibility that carbon tetrachloride increased the sensitivity of the test animal to the drugs. Hence, this question is not pertinent to the problem under investigation. Secondly, the ED50s of barbital sodium in hepatectomized animals tended to be lower at the longer time intervals than ED50s determined in normal rats (figure 6, page 34). This decrease suggests that the operative procedure might have altered in some way either the ability of the animals to excrete barbital sodium or increase their sensitivity to the drug. Studies on the effect of hepatectomy on the renal excretion of barbital sodium were not included in this investigation. A critical comparison of the ED50s for barbital sodium determined in liver-damaged rats (table 2, page 19) and those for this drug determined in hepatectomized rats (table 5, page 30) reveals no significant difference at any of the three time intervals. Thus, it appears that hepatectomy does not alter significantly the sensitivity of the animal as measured by the tests employed.

With regard to bilateral nephrectomy, the ED50s for barbital sodium determined 4 and 8 hours after drug administration were not significantly

different from the ED50s determined in control animals. In contrast, the ED50 of barbital sodium in nephrectomized rats 12 hours after drug administration was only 54 per cent of the ED50 for normal animals. These observations suggest that the rate of absorption of barbital sodium is similar in both nephrectomized and normal rats. It further suggests that peak brain concentrations of barbital sodium are reached before renal excretion significantly lowers the blood level. Thus, the effect of the kidneys on the renal excretion of barbital sodium is most prominent at the longest time interval.

The rate of inulin clearance was slightly decreased (albeit not significant) and barbital excretion was significantly decreased in carbon tetrachloride-treated animals. Although these possibilities were not anticipated when the original hypothesis was formulated, they suggest that carbon tetrachloride administration decreases glomerular function. The decrease, however, is not sufficient to invalidate the use of carbon tetrachloride-induced liver damage as a biological procedure for the study of the fate and excretion of anticonvulsant drugs.

VII. SUMMARY AND CONCLUSIONS

Carbon tetrachloride-induced liver damage and nephrectomy are commonly used to determine the fate and excretion of anticonvulsant drugs. It is generally assumed that such alterations do not otherwise affect the response of the central nervous system to such drugs or to the tests employed to measure their activity. In order to test this assumption, the effects of carbon tetrachloride-induced liver damage and of nephrectomy on the pattern of maximal electroshock seizures (MES) and on the anticonvulsant activity of drugs with known metabolic fates were determined. The effective dose fifty (ED50) of barbital sodium (excreted by the kidney) and of pentobarbital sodium (detoxified by the liver) was determined in rats at various time intervals after drug administration by the MES test (150 mA, 0.2 sec., a.c., corneal electrodes). The effects of carbon tetrachloride on MES pattern and on the anticonvulsant activity of the two drugs tested were determined 48 hours after the subcutaneous injection of carbon tetrachloride (2 ml./kgm. of a 50 per cent w/v solution in peanut oil). The effects of partial hepatectomy and bilateral nephrectomy on MES pattern and on the anticonvulsant activity of the two barbiturates were determined 12 hours after the surgical procedures were performed. The effects of administration of carbon tetrachloride on glomerular filtration rate (measured by inulin clearance) and on the excretion of barbital sodium were also studied. The results were analyzed for statistical significance and may be summarized as follows:

1. Carbon tetrachloride-induced liver damage had no significant effect either on the MES pattern or on the ED50s of barbital sodium, but

significantly decreased the ED50s of pentobarbital sodium at all time intervals studied.

2. Partial hepatectomy altered slightly the MES pattern, but had no significant effect on the ED50s of barbital sodium. In contrast, the ED50s of pentobarbital sodium were significantly decreased at all time intervals studied.

3. Bilateral nephrectomy had no significant effect either on the MES pattern or on the ED50s of pentobarbital sodium, but significantly decreased the ED50 of barbital sodium by the 12-hour interval.

4. Carbon tetrachloride administration did not significantly alter glomerular filtration rate, as measured by inulin clearance. On the other hand, barbital sodium excretion was significantly prolonged in treated animals.

It is concluded that carbon tetrachloride-induced liver damage and nephrectomy provide useful methods for the study of the fate and excretion of anticonvulsant drugs in rats.

VIII. TECHNICAL PROCEDURES EMPLOYED

1. Determination of Inulin in Plasma and Urine (Young and Raisz, 1952)

a. Reagents

(1) Inulin Solution. Dilute 5 ml. of a stock solution of inulin (1000 ugm./ml.) to 50 ml. with distilled water. The diluted solution contains inulin in a concentration of 100 ugm./ml. and is used as a working standard.

(2) Zinc Sulfate Solution, 2 per cent (w/v).

(3) Sodium Hydroxide Solution, 0.1 N and 4.0 N.

(4) Anthrone Solution. Carefully pour 500 ml. of concentrated sulfuric acid into 250 ml. of water and cool the solution to room temperature. Immerse the solution and container in cold water to avoid overheating, and add 250 ml. of concentrated sulfuric acid which contains 4.0 Gm. of dissolved anthrone crystals. Store in brown bottles which are wrapped in heavy brown paper and then dipped in paraffin. If kept away from light this solution will remain stable up to three months.

b. Procedure

Standards

Pipette 0, 1, 2, 3, 4, and 5 ml. of inulin solution into each of six Erlenmeyer flasks (25 ml.), respectively. To each flask add a sufficient quantity of distilled water to bring the total volume to 6 ml.; then add 2 ml. of zinc sulfate solution and 2 ml. of 0.1 N sodium hydroxide solution.

Plasma

Pipette approximately 0.2 ml. of plasma (the exact volume depends upon the quantity of plasma in the capillary tube) into an Erlenmeyer flask (25 ml.). To the flask add a sufficient quantity of distilled water to bring the total volume to 3 ml.; then add 1 ml. of zinc sulfate solution and 1 ml. of 0.1 N sodium hydroxide solution.

Urine

Place a urine sample collected over an approximate 20-minute period (with washings) in an Erlenmeyer flask (25 ml.). To the flask add a sufficient quantity of distilled water to bring the total volume to 6 ml.; then add 2 ml. of zinc sulfate solution and 2 ml. of 0.1 N sodium hydroxide solution.

Agitate the above samples on an automatic shaker for 30 minutes, transfer the solutions to centrifuge tubes, and centrifuge for 15 minutes at 1000 r.p.m. Decant the supernatant liquids. Except for the urine supernatant, the liquids are used undiluted. Dilute the urine supernatant liquid 1:25. Pipette 1 ml. samples of each liquid into 25 x 150 mm. test tubes and add 0.25 ml. of 4 N sodium hydroxide solution to each preparation. Cover each test tube with a glass marble and heat the tubes in a boiling water bath for 20 minutes to destroy any glucose present; then immerse the tubes in cold water. When cool, add 8 ml. of anthrone solution slowly to each test tube.

During the addition of this acid solution, swirl the tube in an ice-water bath to dissipate the heat of dilution and prevent color development, and to insure uniform mixing. Again cover the test tubes with the glass marbles and heat them in a water bath at 75° C ± 0.5° for exactly 5 minutes; the immediately immerse the tubes in cold water. Transfer the cooled solutions to standardized cuvettes and determine the per cent light transmittance by means of a Coleman Junior Spectrophotometer at a wave length of 630 millimicrons. The standard curve for inulin is linear when plotted as a function of the optical density (2-log G, where G equals the per cent light transmittance) versus concentration. The color is stable at room temperature for 4 hours.

2. Determination of Barbiturates in Urine (Goldbaum, 1952)

a. Solvent

Wash ten parts of reagent grade chloroform once with one part of approximately 1 N sodium hydroxide and twice with one part of water. Prepare only the volume required for immediate use. The chloroform tends to decompose on standing and, thus, should be washed every day.

b. Reagents

(1) Monobasic Potassium Phosphate Solution 0.2 M (U. S. P. XV)

Dissolve 27.218 Gm. of monobasic potassium phosphate (KH_2PO_4) in sufficient double-distilled water to measure 1000 ml.

(2) Sodium Hydroxide Solution 0.2 M (U. S. P. XV)

Dissolve 9 Gm. of sodium hydroxide in about 950 ml. of double-distilled water. Add a freshly prepared saturated solution of barium hydroxide until no more precipitate forms. Shake the mixture thoroughly, and allow it to stand overnight in a stoppered bottle. Either decant the clear liquid or filter the solution, then standardize it against 1 N hydrochloric or sulfuric acid using two drops of phenolphthalein T. S. as an indicator. Titrate to a pink color. Preserve this solution in a well-filled Pyrex bottle with a soda-lime tube attached to the bottle by a glass side arm.

(3) Phosphate Buffer pH 7.4 (U. S. P. XV)

Place 50 ml. of the monobasic potassium phosphate solution (see (1) above) in a 200 ml. volumetric flask, add 39.34 ml. of the sodium hydroxide solution (see (2) above), and add double-distilled water to the mark.

(4) Borate Buffer

Dissolve 12.369 Gm. of boric acid and 14.911 Gm. of potassium chloride in double-distilled water and dilute to 200 ml. After standing at room temperature for 24 hours, filter off any undissolved salts.

(5) Sodium Hydroxide Solution

Prepare an approximately 0.45 N sodium hydroxide solution from a saturated solution of sodium hydroxide. Adjust the normality until two parts of this solution and one part of

borate buffer yield a solution with pH 10.5 when tested with an accurately calibrated pH meter.

c. Procedure

Dilute the urine sample 1:25, 1:50, or as necessary with distilled water in order to bring the expected concentration of barbiturate within a readable range on the spectrophotometer. Extract 1 to 5 ml. of diluted urine (pH below 7.0) with 50 ml. of chloroform. To remove interfering substances, transfer the chloroform layer to a clean separatory funnel and shake with 5 ml. of phosphate buffer pH 7.4. Discard the phosphate layer. To obtain a clear aliquot, filter the chloroform through a Whatman no. 41 filter paper. Extract the filtrate with 4 ml. of approximately 0.45 N sodium hydroxide in a dry separatory funnel. Discard the chloroform layer and run the alkaline solution into a small test tube and centrifuge. Transfer 3 ml. of the clear extract to a 1 cm. quartz cuvette of a Beckman model DU photoelectric spectrophotometer. Run a blank of distilled water through the same extraction procedure and use it as a reference solution. Read the optical density of the extracts at wave lengths of 305, 270, 260, 252, 247, 235, 232, and 228 mu. Pipette 2 ml. of the extract of the urine sample and of the reference solution into test tubes containing 1 ml. of 1 M boric acid-potassium chloride buffer solution to yield a pH of 10.5; the pH must be below 10.6 and above 10.2. Transfer the buffered solutions to dry cuvettes and again determine the optical densities at the above wave lengths. Correct these optical densities for dilution

with the buffer by multiplying by 1.5. The difference between the two readings at 260 mu. is termed the optical density difference. Determine the optical density difference of a known quantity of barbiturate in a similar manner. Estimate the concentration of barbiturate in the unknown sample from the optical density differences of the unknown and known samples of barbiturate.

d. Calculation of Barbiturate Concentration

Calculate the concentration of barbiturate in urine, expressed as mgm. of the barbiturate excreted per kgm. of animal weight, as shown in the following example:

(1) Difference of unknown extract unbuffered and buffered at 260 mu. = 0.216.

(2) Volume of chloroform used to extract the sample = 50 ml.

(3) Volume of alkali solution used to extract chloroform aliquot = 4 ml.

(4) Micrograms of barbital sodium per ml. of known extract = 25.

(5) Difference of known extract unbuffered and buffered at 260 mu. = 0.261.

(6) Chloroform aliquot = 47 ml.

(7) Volume of sample = 1 ml.

(8) Dilution factor = 25.

(9) Total urine output for 4-hour period = 5.4 ml.

(10) Total rat weight = 1.080 kgm.

(11) Micrograms of barbital sodium per ml. of sample = $\frac{(1)(2)(3)(4)}{(5)(6)(7)}$

$= \frac{(0.216)(50)(4)(25)}{(0.261)(47)(1)} = \frac{1080.000}{12.267} = 88.041.$

(12) Micrograms of barbital sodium per ml. of urine = (11)(8) = (88.041)(25) = 2201.025.

(13) Total micrograms of barbital sodium in urine sample = (12)(9) = (2201.025)(5.4) = 11885.535.

(14) Milligrams of barbital sodium excreted per kilogram of rat weight = $\frac{(13)}{(10)} \times \frac{1}{1000} = \frac{(11885.535)}{(1.080)} \times \frac{1}{1000} = 11.005$.

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VALIDITY OF CARBON TETRACHLORIDE-INDUCED LIVER DAMAGE AND NEPHRECTOMY AS BIOLOGICAL PROCEDURES FOR DETERMINING FATE AND EXCRETION OF ANTICONVULSANT DRUGS

by

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MASTER OF SCIENCE

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Carbon tetrachloride-induced liver damage and nephrectomy are commonly used to determine the fate and excretion of anticonvulsant drugs. It is generally assumed that such alterations do not otherwise affect the response of the central nervous system to such drugs or to the tests employed to measure their activity. In order to test this assumption, the effects of carbon tetrachloride-induced liver damage and of nephrectomy on the pattern of maximal electroshock seizures (MES) and on the anticonvulsant activity of drugs with known metabolic fates were determined. The effective dose fifty (ED50) of barbital sodium (excreted by the kidney) and of pentobarbital sodium (detoxified by the liver) was determined in rats at various time intervals after drug administration by the MES test (150 mA, 0.2 sec., a.c., corneal electrodes). The effects of carbon tetrachloride on MES pattern and on the anticonvulsant activity of the two drugs tested were determined 48 hours after the subcutaneous injection of carbon tetrachloride (2 ml./kgm. of a 50 per cent w/v solution in peanut oil). The effects of partial hepatectomy and bilateral nephrectomy on MES pattern and on the anticonvulsant activity of the two barbiturates were determined 12 hours after the surgical procedures were performed. The effects of administration of carbon tetrachloride on glomerular filtration rate (measured by inulin clearance) and on the excretion of barbital sodium were also studied. The results were analyzed for statistical significance and may be summarized as follows:

1. Carbon tetrachloride-induced liver damage had no significant effect either on the MES pattern or on the ED50s of barbital sodium, but

significantly decreased the ED50s of pentobarbital sodium at all time intervals studied.

2. Partial hepatectomy altered slightly the MES pattern, but had no significant effect on the ED50s of barbital sodium. In contrast, the ED50s of pentobarbital sodium were significantly decreased at all time intervals studied.

3. Bilateral nephrectomy had no significant effect either on the MES pattern or on the ED50s of pentobarbital sodium, but significantly decreased the ED50 of barbital sodium by the 12-hour interval.

4. Carbon tetrachloride administration did not significantly alter glomerular filtration rate, as measured by inulin clearance. On the other hand, barbital sodium excretion was significantly prolonged in treated animals.

It is concluded that carbon tetrachloride-induced liver damage and nephrectomy provide useful methods for the study of the fate and excretion of anticonvulsant drugs in rats.